DRG® Cartilage Oligomeric Matrix Protein (Human) (EIA-5265)

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This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Please use only the valid version of the package insert provided with the kit.

1 INTENDED USE

The Human Cartilage Oligomeric Matrix Protein ELISA is a sandwich enzyme immunoassay for measurement of human cartilage oligomeric matrix protein (COMP).

Features

The total assay time is less than 3.5 hours

The kit measures COMP in serum and plasma (EDTA, citrate, heparin)

Assay format is 96 wells

Quality Controls are human serum based

Standard is recombinant protein based

Components of the kit are provided ready to use, concentrated or lyophilized

2 STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

3 TEST PRINCIPLE

In the Human Cartilage Oligomeric Matrix Protein ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human COMP antibody. After 60 minutes incubation and washing, biotin labelled second monoclonal anti-human COMP antibody is added and incubated with captured COMP for 60 minutes. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of COMP. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

4 PRECAUTIONS

For professional use only

Wear gloves and laboratory coats when handling kit materials

Do not drink, eat or smoke in the areas where kit materials are being handled

This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents

This kit contains components of animal origin. These materials should be handled as potentially infectious

Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary

The materials must not be pipetted by mouth

5 TECHNICAL HINTS

Reagents with different lot numbers should not be mixed

Use thoroughly clean glassware

Use deionized (distilled) water, stored in clean containers

Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent

Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light

Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution

Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

6 REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody	ready to use	13 mL
Streptavidin-HRP Conjugate	ready to use	13 mL
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	2 x 13 mL
Wash Solution Conc. (10x)	concentrated	100 mL
Substrate Solution	ready to use	13 mL
Stop Solution	ready to use	13 mL
Product Data Sheet + Certificate of Analysis	-	1 pc

7 MATERIAL REQUIRED BUT NOT SUPPLIED

Deionized (distilled) water
Test tubes for diluting samples
Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
Precision pipettes to deliver 5-1000 µL with disposable tips
Multichannel pipette to deliver 100 µL with disposable tips
Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
Vortex mixer
Orbital microplate shaker capable of approximately 300 rpm
Microplate washer (optional). [Manual washing is possible but not preferable.]
Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
Software package facilitating data generation and analysis (optional)

8 PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use

Always prepare only the appropriate quantity of reagents for your test

Do not use components after the expiration date marked on their label

Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2 °C - 8 °C and protected from the moisture.

Biotin Labelled Antibody
Streptavidin-HRP Conjugate
Dilution Buffer
Substrate Solution
Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2 °C - 8 °C.

Assay reagents supplied concentrated or lyophilized:

Human COMP Master Standard

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Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of the COMP in the stock solution is **128 ng/mL**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	128 ng/mL
300 µL of stock	300 µL	64 ng/mL
300 µL of 64 ng/mL	300 µL	32 ng/mL
300 µL of 32 ng/mL	300 µL	16 ng/mL
300 µL of 16 ng/mL	300 µL	8 ng/mL
300 µL of 8 ng/mL	300 µL	4 ng/mL

Prepared Standards are ready to use, do not dilute them.

Stability and storage:

Do not store the Standard stock solution and set of standards.

Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!

Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:

The reconstituted Quality Controls must be used immediately or stored frozen at -20 °C for 1 month. Avoid repeated freeze/thaw cycles.

Note:

Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with the user's manual and Certificate of Analysis and that ELISA test was carried out properly.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 mL of Wash Solution Concentrate (10x) + 900 mL of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2 °C - 8 °C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2 °C - 8 °C.

9 PREPARATION OF SAMPLES

The kit measures human COMP in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20 °C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples **50x** with Dilution Buffer just prior to the assay (e.g. 5 µL of sample + 245 µL of Dilution Buffer for singlets or duplicates). **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles.

Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at 2 °C - 8 °C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of human COMP.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

10 ASSAY PROCEDURE

1. Pipet **100 µL** of Standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25 °C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 mL per well).
   After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µL** of Biotin Labelled Antibody into each well.
5. Incubate the plate at room temperature (ca. 25 °C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 mL per well).
   After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µL** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25 °C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 mL per well).
   After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µL** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.

11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20 °C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µL** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm.
    The absorbance should be read within 5 minutes following step 12.

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine COMP concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were "in range" at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 mL Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 128	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 64**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 32**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 16**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 8**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 4**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC HIGH**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC LOW**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

11 CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of COMP ng/mL in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve, i.e. *logit* of the mean absorbance (Y) is plotted against *log* of the known concentration (X) of Standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 20 ng/mL (from standard curve) x 50 (dilution factor) = 1 000 ng/mL.

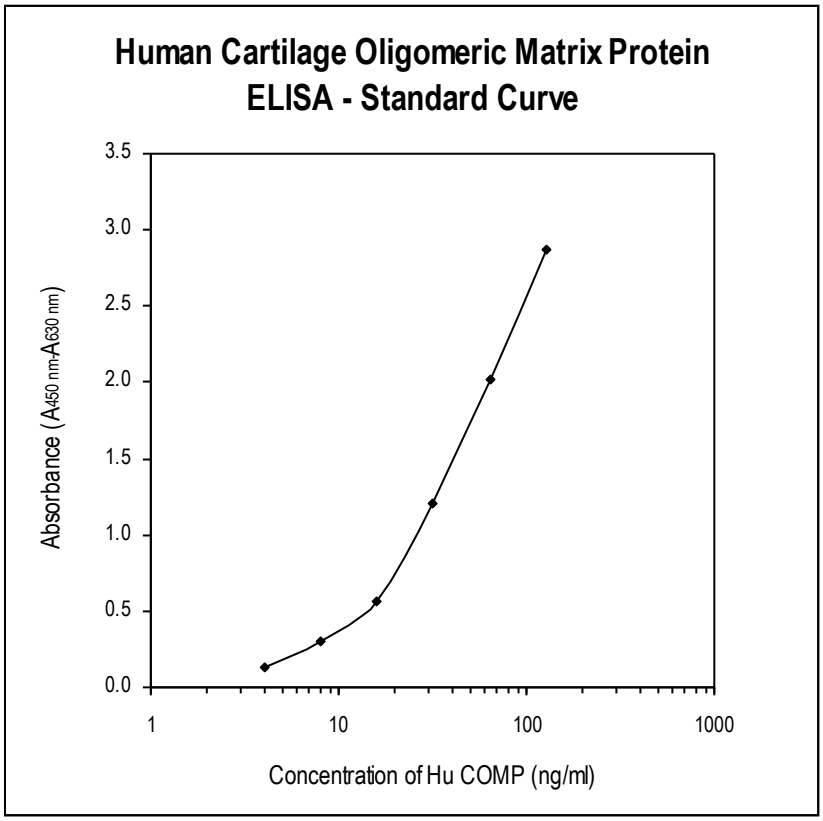

Figure 2: Typical Standard Curve for Human Cartilage Oligomeric Matrix Protein ELISA.

12 STABILITY OF SAMPLES STORED AT 2 °C - 8 °C

Samples should be stored at –20 °C. However, no decline in concentration of COMP was observed in serum and plasma samples after 7 days when stored at 2 °C - 8 °C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp, Period	Serum (ng/mL)	Plasma (ng/mL)		
			EDTA	Citrate	Heparin
1	-20 °C	443	328	352	488
	2 °C - 8 °C, 1 day	481	302	355	499
	2 °C - 8 °C, 7 days	482	264	345	374
2	-20 °C	985	674	711	910
	2 °C - 8 °C, 1 day	993	631	728	837
	2 °C - 8 °C, 7 days	1 024	601	771	1 036
3	-20 °C	847	491	629	713
	2 °C - 8 °C, 1 day	866	515	654	793
	2 °C - 8 °C, 7 days	905	493	605	853

12.1 Effect of Freezing/Thawing

No decline was observed in concentration of human COMP in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (ng/mL)	Plasma (ng/mL)		
			EDTA	Citrate	Heparin
1	1x	1 105	632	820	1 121
	3x	1 040	610	783	914
	5x	1 010	559	727	867
2	1x	942	702	733	921
	3x	893	665	707	764
	5x	894	716	614	728
3	1x	1 224	884	1 133	1 100
	3x	1 275	843	975	1 182
	5x	1 220	856	873	1 080

13 DEFINITION OF THE STANDARD

The recombinant human COMP is used as the Standard. The recombinant human COMP is produced in cell line HEK293.

13.1 Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological references ranges for COMP levels with the assay.

14 TROUBLESHOOTING AND FAQS

- **Weak signal in all wells**

Possible explanations:

Omission of a reagent or a step
Improper preparation or storage of a reagent
Assay performed before reagents were allowed to come to room temperature
Improper wavelength when reading absorbance

- **High signal and background in all wells**

Possible explanations:

Improper or inadequate washing
Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
Incubation temperature over 30 °C

- **High coefficient of variation (CV)**

Possible explanation:

Improper or inadequate washing
Improper mixing Standards, Quality Controls or samples

15 REFERENCES

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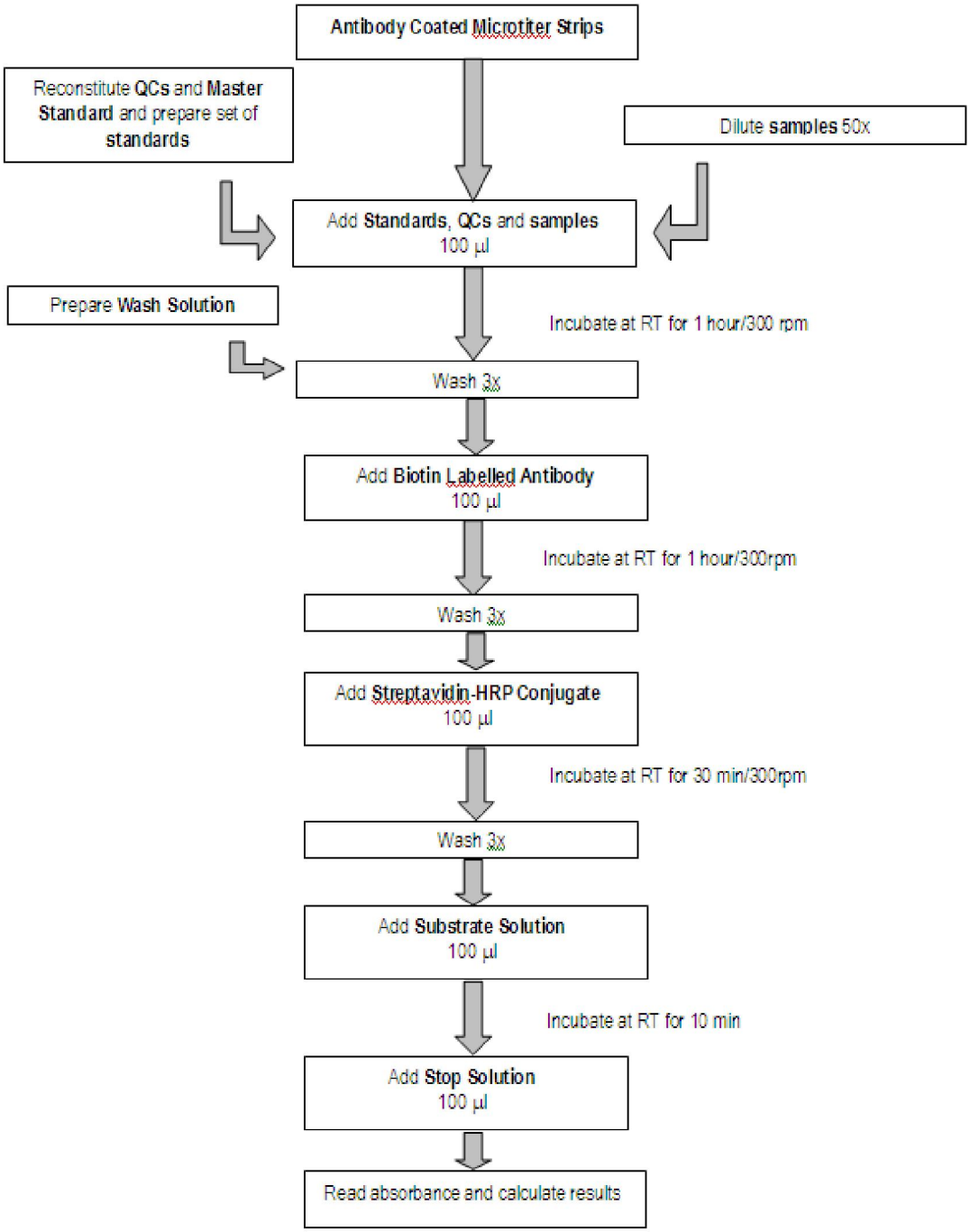
16 ASSAY PROCEDURE SUMMARY


Antibody Coated Microtiter Strips
Reconstitute QCs and Master Standard and prepare set of standards
Dilute samples 50x
Add Standards, QCs and samples 100 µl
Prepare Wash Solution
Incubate at RT for 1 hour/300 rpm
Wash 3x
Add Biotin Labelled Antibody 100 µl
Incubate at RT for 1 hour/300rpm
Wash 3x
Add Streptavidin-HRP Conjugate 100 µl
Incubate at RT for 30 min/300rpm
Wash 3x
Add Substrate Solution 100 µl
Incubate at RT for 10 min
Add Stop Solution 100 µl
Read absorbance and calculate results

CE

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